ISSN: 0975 – 8704
IJBLST (2017), 9(3):18-25

Bio-ethanol yielding potentials of Melon seed peels using fungal isolates from Palm oil effluents

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ABSTRACT:
In this study, the feasibility of fermenting melon seed peels for bio-ethanol production was investigated under standard laboratory conditions using *Aspergillus niger, Neurospora crassa* and *Saccharomyces cerevisiae* isolated from palm oil effluents. The melon seed wastes were collected from the dump sites, processed and hydrolyzed using dilute mineral acid. One hundred milliliters (100ml) portion of the sterilized hydrolysate was then fermented using the fungal isolates as axenic and mixed cultures at temperature at various temperature (25-50^{0}C and pH (4.0-5.0). Bio-ethanol production was monitored periodically from the treated and untreated substrates for a period of 10 days. The total reducing sugar contents obtained from the treated substrates was 32.05%. Maximum ethanol yield of 10.5 % v/v was obtained by the mixed culture at day 6, while *A. niger, N. crassa* and *S. cerevisiae* singly gave a peak ethanol yields of 7.5%, 5.2% and 7.9% v/v after 6 days of fermentation. Temperature and pH had significant ($P<0.05$) effect on bio-ethanol yield, with the optimal range being 35 ± 2^{0}C, and 4.4-5.0 respectively. The findings from this study suggest that melon seed peels, a common agro-waste, contain fermentable sugars that could be exploited for bio-ethanol production using palm oil effluent fungi

Key words: Bio-fuel, Melon seed peel, palm oil effluent, fossil fuel

INTRODUCTION

Bioethanol is a renewable energy source produced mainly by the microbiological fermentation of sugar sources. Considering the dwindling oil reserves, soaring oil prices and negative environmental effects of oil pollutions, there is an increasing demand for bioethanol production as feedstock and more importantly as an alternative source of liquid fuel for automobiles globally [1-3]. The high prices have led to energy crises in both developing and developed countries that utilize large quantity of oil for domestic and industrial purposes.

In recent years, the usefulness of biofuels such as biogas, bioethanol have increased globally, and recently, most African nations such as Nigeria had keyed into the idea of biofuel production with the aim of generating wealth [4]. The main usefulness of bioethanol is the possibility to blend it gasoline for use in engines without any significant change in internal combustion. By blending ethanol with gasoline we can also oxygenate the fuel mixture so it burns more completely and reduces pollution emission. The most common blend is 10% ethanol and 90% petrol (E10) which requires no modification of vehicle engines to run it, and also no effect on vehicle warranties [5]. Only flexible fuel vehicles can run on up to 85% ethanol and 15% petrol blends (E85) [6]. Ethanol fuel blends are widely sold in the United States of America

One of the major processes of producing ethanol, apart, from synthesized by chemical processes such as reacting ethylene with steam [7], is through the fermentation of starchy or sugar crop such as sugarcane, sugar beet, sweet sorghum, yam, sweet potato, corn and cassava which are some of the main starches that serve as staple foods for people through the world's hot and humid regions [8, 9]. The major drawback of this process is that most of these crops are staple food crops and tend to increase the cost of production. The diversion of food resource to biofuel production may to a large extent have fuelled the current food crises worldwide [10].

In order to make the fermentation method cost effective and to meet the great demand for ethanol, attention have been shifted to the generation of bioethanol and other biofuels from cheaper raw materials as well as the study of new microorganisms or yeast strains efficient in ethanol production [1, 9, 11-14]. It, therefore, becomes imperative that the searchlight be turned at present to the use of non-food starchy inexpensive raw

ISSN: 0975 – 8704
IJBLST (2017), 9(3):18-25

materials such as agricultural and industrial wastes to produce ethanol cheaply.

Agricultural wastes, including wood, herbaceous plants, crops and forest residues, and other animal waste have been known to serve as immense source of bio-energy. These wastes are generated annually in large amounts and are vastly underutilized. The practice is usually to burn them or leave them to decompose. Studies have shown that these biomass residues could be processed into liquid fuel such as biogas and bioethanol, or combusted to produce electricity and heat [2]. This reduction of greenhouse gas emission and other negative environmental effects are the main advantages of utilizing biomass conversion into ethanol [7, 15, 16]. Several agricultural wastes are currently considered as great potential substitute for fossil-fuel-based energy resources [17-23].

Melon (*Colocynthis citrullus* L.) is a widely cultivated annual crop mainly for its seeds from which a number of products could be obtained. The seeds are edible and consumed in virtually all parts of Nigeria and in many other parts of the world [24]. In Nigeria, the seeds are processed daily into food condiments (Ogiri), cake and other special delicacies because of their high nutritional content [25]. The processing of melon to get the seeds involves manual or mechanical separation or shelling of the seed to obtain the required edible pulp. This has led to the generation of large quantity of melon seed shell wastes which are dumped indiscriminately without recourse to their health and environmental implications. In an attempt to derive wealth from these wastes, this study was therefore undertaken to assess its bio-ethanol generation capacity. Currently, there is paucity of information on the suitability of melon seed peel wastes in bio-ethanol production. This study was therefore undertaken to evaluate the suitability of using the peel wastes of melon seeds for bio-ethanol production using common palm oil effluent fungal isolates.

MATERIALS AND METHODS

Source of melon seed peels

Melon seed shells were collected from a dump site close to markets and residential areas in Amai and Obiaruku, located in Ukwuani Local Government Area of Delta State, Nigeria, and immediately transported in a clean polyethylene bag to the Microbiology laboratory for further analysis. The samples were washed with sterile water, air dried for a week and reducedto smaller particles using a Waring Blender (Binatone).

Melon seed

Isolation of fungi from palm oil effluent

Palm oil effluent was collected from dump sites around palm oil milling center along Amai-Ogume road and transported in sterilized bottle to the laboratory for microbial analysis. The sample was serially diluted, and 1 ml cultured via pour-plate techniques on sterile Malt yeast extract agar and Potato Dextrose Agar plates (supplemented with 0.1% lactic acid). The plates were incubated at ambient temperature (30± 2^{o}C) for 2-4days. The predominant discrete colonies were purified, stained with lactophenol cotton blue stain and confirmed under the microscope and later characterized based on their morphological, cultural and biochemical sugar tests as described earlier [26, 27].

Hydrolysis of substrate

Two hundred grams of the pre-treated substrate (by boiling in water) was weighed into 500ml Erlenmeyer flask containing 50 ml of distilled water. The flask content was thoroughly mixed were hydrolyzed with 0.6M H_2SO4 at 90^{o}C in a water bath for 30 minutes. The flask was allowed to cool, filtered through No. 1 Whatman filter paper and the pH of the hydrolysate was adjusted to 4.5 with 0.4 M NaOH. The broth filtrate was used to determine the level of reducing sugars in the substrate.

ISSN: 0975 – 8704
IJBLST (2017), 9(3):18-25

Table 1: Major Characteristics of the predominant fungi isolates of palm wine

Isolate Code	Cultural /Macroscopic Characteristics	Fungi	% Occurrence
PWF1	Black mycelia hyphal growth	*Aspergillus niger*	25 (54%)
PWF2	Woolly-like whitish mycelia growth	*Neurospora crassa*	9 (20%)
PWF3	Creamy, white coloured, smooth colonies	*Sacharomyces cerevisiae*	12 (26%)

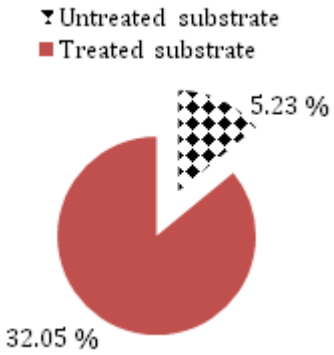

Fig 1: Total reducing sugar content of treated and untreated substrate

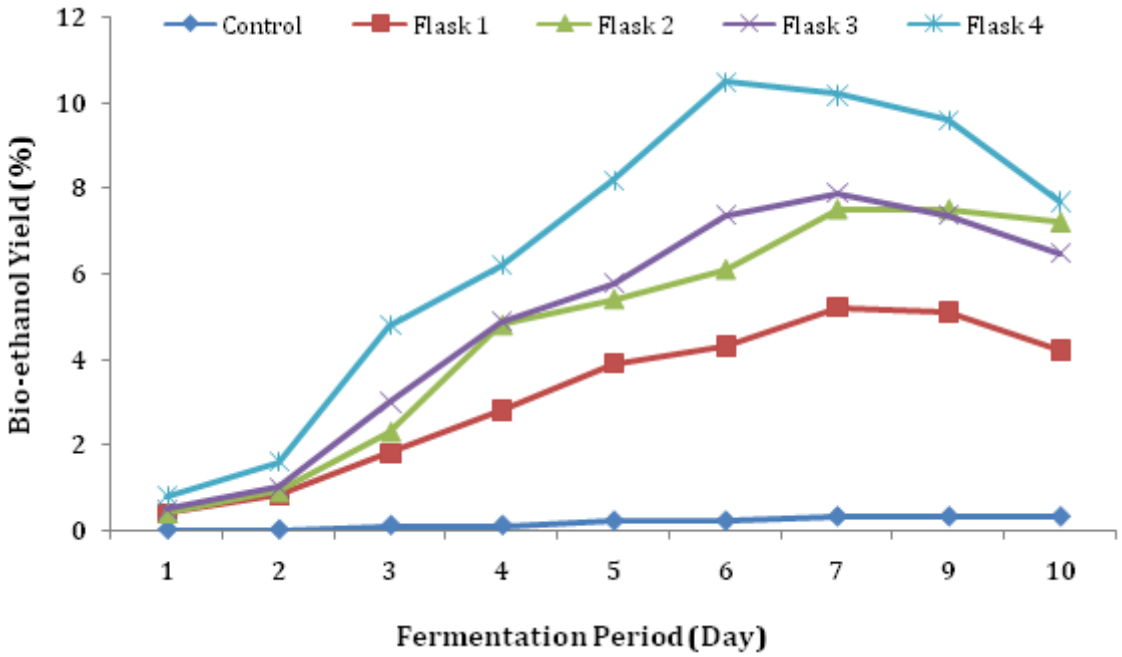

Fig 2: Bioethanol yield from fermented melon peel waste hydrolystae (%l)
Control flask = Only substrate; **Flask 1** = Substrate + *N. crassa;* **Flask 2** = Substrate + *A. niger;* **Flask 3** =Substrate + *S. cersvisaie*; **Flask 4** = Substrate + *N. crassa* + *A. niger* + *S. cerevisiae*

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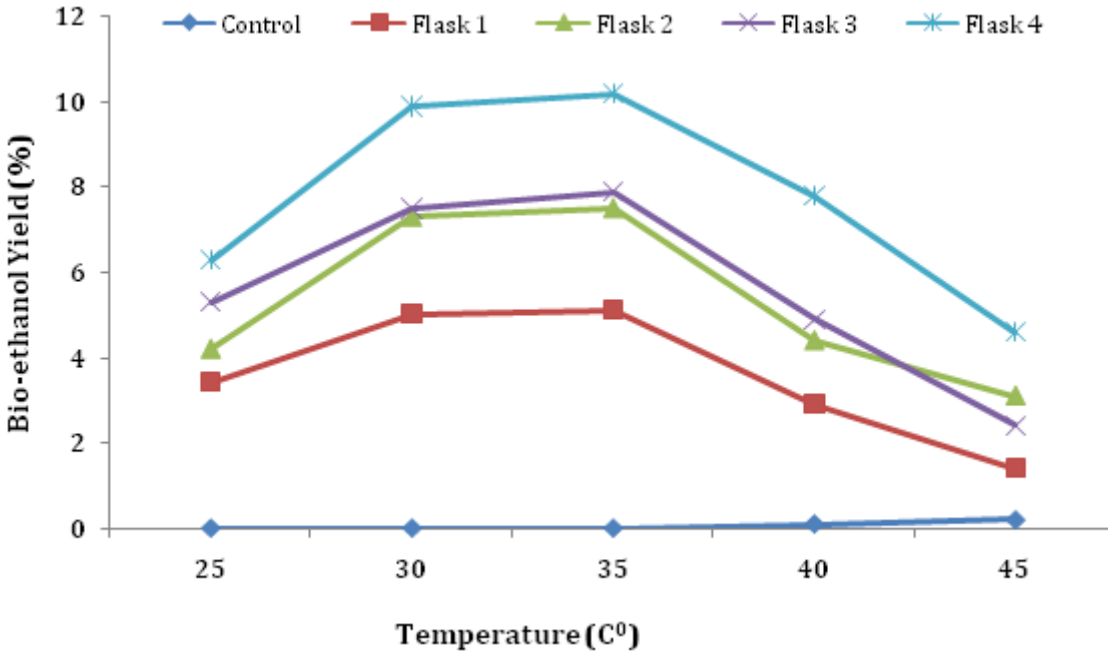

Figure 3: Effect of Temperature on maximum bio-ethanol yield (%)

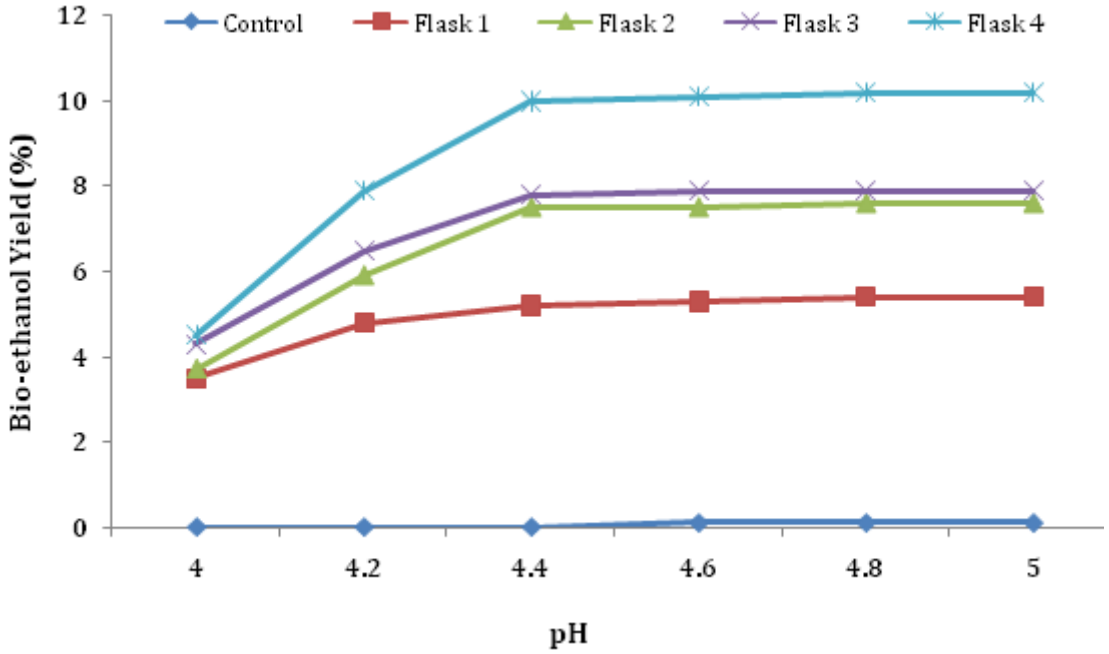

Fig. 4: Effect of pH on maximum bio-ethanol yield (%)

ISSN: 0975 – 8704
IJBLST (2017), 9(3):18-25

Determination of Reducing Sugar
The reducing sugar concentration following hydrolysis of the melon peel material was analyzed using the Dinitrosalicylic acid (DNS) method and absorbance determined at 540nm using JENWAY 6400 spectrophotometer [28]. The reducing sugar content of the sample was subsequently determined by making reference to a standard curve of known glucose concentrations.

Fermentation of Hydrolysate
The modified method of Brook [1] was applied. 100g portion of the hydrolysate was weighed and dispensed into seven 500ml conical flask containing 100 cm^3 distilled water supplemented with 10g/l yeast extract (5g/l) and petptone (10g/l). Each flask was replicated seven times. The flasks were the covered with cotton wool, wrapped in aluminum foil, autoclaved at 121^0C for 15 minutes. and allowed to cool at room temperature. There were aseptically inoculated with 2 ml suspension of starter culture (OD=0.6) of *N. crassa, A. niger* and Saccharomyces *cerevisiae* singly and in equal mixture. The control lacks the starter cultures. All the flasks were monitored ethanol production every 24 hrs for 10 days under adjusted temperature ranges of 25-50^0C and pH ranges of 4.0-5.0

Distillation
This was done using distillation apparatus (set up). The different fermenting broth was transferred into round bottom flask and placed on a heating mantle fixed to a distillation column enclosed in running tap water. Another flask was fixed to the other end of distillation column to collect the distillate at 78^0C (standard temperature for ethanol production).

Determination of Ethanol Concentration
Ethanol concentration was determined by comparing the density of the ethanol produced with the standard ethanol density curve. Standard ethanol curve was prepared by taking series of percentage (v/v) ethanol (10%, 20%, 30%, 40% and 50%) solution in a 100ml volumetric flask and the weights measured as described earlier [28]. The density of each of the prepared ethanol solution was calculated and a standard curve of density against percentage ethanol (v/v) was plotted from which the bio-ethanol concentrations were extrapolated.

RESULTS AND DISCUSSION
The characterized fungal isolates from palm oil effluents were *Aspergillus niger*, *Neurospora crassa* and *Sacharomyces cerevisiae* (Table 1). These fungal were predominant in the effluent samples analysis. They were used for the study because previous research have shown that they to possess efficient enzymatic machinery needed break down complex polysaccharides found in plant materials [9, 18, 23, 30, 31, 33].

The results of the dilute acid hydrolysis of the pre-boiled melon seed peel samples are presented in Figure 1. From the results, it was observed that the total reducing sugar content of the substrate was 32.05%. This value is relatively significant when compared to the results of reducing sugars obtained from other agro-waste, such as cassava peels, corncobs potatoes peels, banana peels, plantain peels, pineapples peels, rice husks as reported earlier. The reducing sugar concentration of pineapple, banana, and plantain peels ranged between 0.27-0.94mg/cm^3 and 0.20-0.82mg/cm^3 and 0.16-0.45 mg/cm^3 respectively [18]. The valuable amount of reducing sugar obtained from the hydrolysate could be attributed to the initial hot water treatment followed by dilute acid hydrolysis of the samples, which according to Arumugam and Manikandan [35] helps to disintegrates the complex lignocelluloses biomass to fermentable sugar residues. Hence, the need to encourage pre-treatment of the lignocelluloses agro-waste before utilization for bioethanol production. This submission is in consonance with the findings of Ebabhi et al. [22] who reported that high amount of total reducing sugars visa-vice high bioethanol production could be generated by pre-treatment of cellulosic agro-waste with dilute acid/enzymatic hydrolysis.

The results of bio-ethanol production using the axenic and mixed cultures of the fungi are shown in Figure 2. The bio-ethanol yield of the mixed culture was found to peaked at day 6 with a maximum yield of 10.5 % v/v. This was higher than the bioethanol yield from each of the individual fungal culture. For the axenic cultures, a maximum ethanol yield of 7.5%, 5.2% and 7.9% v/v were obtained from fermentation flask containing each of *A. niger, N. crassa* and *S. cerevisiae* singly after day 6. The yields obtained were comparable to the yields reported for Pineapple peels (8,34%), banana peels (7.45%), sugarcane baggasse (6.72%), sugarcane bark (6.23%) and corn stalk (6.17%) [18, 36]. However, the bioethanol yield obtained in this study were relatively higher than those reported for corn cob (4.17%), plantain peel (3.98%), and corn husk

(3.45%) [18, 36]. The slight differences could be attributed to the variations in the agro-waste type and concentration, procedures of substrate pre-treatment, saccharifiction agents, microbial inoculants and duration of fermentation.

The high bioethanol yeild by the mixed fungal culture could be attributed to co-metabolic activities contributed by the individual fungi. This finding is in line with precious workers, who reported that co-culture of fungi demonstrated higher ethanol yield than each monoculture [37]. This result also suggested that there was less competition, antagonism and or predation by the individual fermenting fungal culture that make up the mixed culture. Competition, antagonism and or predation have been reported as the major draw backs in using consortia cultures for biodegradation of organic compounds [38]. Also, the significant bioethanol yield by the individual test fungal culture indicates that each of them possess the requisite fermentative enzymes that can attack cellulose agro waste and generate bioethanol. Comparing the rate of bioethanol yields of the monoculture, the activities demonstrated by *S cerevisiae* is worth-noting because, it produced the highest amount of bioethanol (7.9%), followed by *A. niger* (7.5%) and *N. crassa* (5.2%). *S cerevisiae* have been reported as one of the potential yeast with versatile fermetative enzymes exploitable for diverse biotechnological applications [39]. Also, the efficiency of bioethanol production of *A. niger* and *N. crassa* have been be reported in the past [23-34]. Hence, these fungal could be exploited as bio-agents for bioethanol production.

The optimal temperature range and pH for maximum bioethanol yield were found to be 35 ± 2^0C and 4.4-5.0 respectively (Figure 3 and 4). Temperature and pH are two important physicochemical factors that can affect the enzymatic activities of organisms. The changes in temperature and pH of the fermenting medium in this study were not significantly different from the effects reported by previous workers [29, 30]. Fungi are generally known to tolerate acidic pH that affects the activities of bacterial. Hence, optimizing the appropriate ranges of pH and temperature, greater yield of bioethanol could be obtained from fermenting the substrate using fungi.

CONCLUSIONS

The use of complementary or alternative renewable bio-energy from agro-waste cannot be over-emphasis in the light of their obvious advantages over the conventional system. This study has shown that *S.* cerevisiae, *A. niger* and *N. crassa*, isolated from palm oil effluents could utilized to ferment pre-treated melon seed peel to produce useful bio-ethanol. The rate of bioethanol production was observed to be highest when the three of the fungal culture were mined together as mixed culture for a period 6 days. Each of the fungal culture also demonstrated significant bioethanol production with *S. cerevisiae* yielding the most, followed by *A. niger* and *N. crassa* being the least. This study has therefore shown that melon seed peels, a common agro-waste, contain fermentable sugars could be exploited for bio-ethanol production using palm oil effluent fungal isolates as mixed or individual culture under appropriate temperature and pH conditions. Further study is however required to investigate the factors that could enhance or hamper maximization of bioethanol production potentials from this substrate with a view to considering them for future exploitation.

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ISSN: 0975 – 8704
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